

## References with Coulter *et al.* (1983) citations

	Author	Coulter and Harris (1983) citation in text of article
1	Skurnick S <i>et al.</i> (2010)	Fab fragments purified from the human mAb to PNAG, prepared using papain conjugated to agarose beads (Sigma-Aldrich) as described (40);
2	Backovic M <i>et al.</i> (2010)	Enzymatic digestion of antibodies with papain (and less often with pepsin) has traditionally been used for Fab production (Porter, 1958, 1959; Coulter and Harris, 1983; Rousseaux <i>et al.</i> , 1983).
3	Nasseff HM <i>et al.</i> (2009)	Fab fragments can be directly generated using thiol proteases such as papain or ficin or, alternatively, F(ab') <sub>2</sub> fragments can be generated using bromelain, pepsin, or ficin, and the disulfide hinge is subsequently cleaved using a reducing agent, generating Fab fragments. <sup>9,10</sup>
4	Niwa T <i>et al.</i> (2009)	The concentration of the obtained F(ab) <sub>2</sub> fragments was calculated based on their absorption at 280 nm, assuming an <i>E</i> value of 14 [19].
5	Silva SR <i>et al.</i> (2010)	To obtain Fab and Fc fragments, IgG1 samples were digested by solid phase papain (Sigma-Aldrich), as described by Coulter and Harris (28).
6	Kimura S <i>et al.</i> (2008)	Preparation of Fab fragments was performed as previously described (Coulter and Harris, 1983).
7	Brereton HM <i>et al.</i> (2005)	Fab fragments were purified on a Q Sepharose HP column and eluted with a linear NaCl gradient (0–1 M). <sup>30</sup>
8	Gora M <i>et al.</i> (2004)	Rabbit IgG Fab preparations were prepared using immobilized papain (Perbio Science, Tattenhall, UK) followed by chromatography through protein A Sepharose to remove the undigested IgG and Fc fragments [27].
9	Kobayashi N <i>et al.</i> (2003)	The flow-through solution containing the F(ab') <sub>2</sub> fragment was collected, dialysed against cold buffer A (1 day), concentrated and its protein concentration determined from the absorption at 280 nm assuming an E-value of 14 (Coulter and Harris, 1983).
10	Mukherjee M <i>et al.</i> (2002)	Anti-78 kDa Fab fragments were prepared as described by Coulter and Harris (1983).
11	Luo QZ <i>et al.</i> (2002)	Therefore the optimal incubation time for cleavage of IgG with immobilized papain is much longer than that with free papain, which was consistent with the result obtained by Coulter <i>et al.</i> [27].
12	Kobayashi N <i>et al.</i> (2000)	The flow-through solution containing the F(ab') <sub>2</sub> fragment was collected, and its protein concentration determined from the absorption at 280 nm assuming an E-value of 14 (Coulter and Harris, 1983).
13	Mullock BM <i>et al.</i> (2000)	Monovalent Fab fragments of rabbit anti-Syn7#2 IgG were prepared according to Coulter and Harris (1983) and then affinity purified like the intact antibodies.
14	Gomez HF <i>et al.</i> (1999)	The IgG was purified using a modification of the method described by Goding <sup>24</sup> and Coulter <i>et al.</i> <sup>25</sup>
15	Clement S <i>et al.</i> (1999)	Fab fragments were prepared by a slight modification of the method of Coulter and Harris. <sup>21</sup>
16	Almeida SR <i>et al.</i> (1998)	The product was passed once through a protein-A-Sepharose column. F(ab) fragments were obtained in the void volume, whereas intact IgG and Fc fragments were retained in the column [18].
17	Sinha D <i>et al.</i> (1998)	To obtain Fab fragments, the antibodies were cleaved with papain (Coulter and Harris, 1983) and passed over a protein A column to remove the Fc fragments and any undigested IgG.
18	Ng PC <i>et al.</i> (1997)	The protein concentration of IgG, F(ab') <sub>2</sub> , and Fab was determined from the absorbance at 280 nm with a Beckman DU640 spectrophotometer (Beckman Instruments, Inc., Fullerton, California USA) using the extinction coefficient of 1.4 (cm • mg/ml). <sup>15</sup>
19	Koyama H <i>et al.</i> (1996)	Fab fragments of monoclonal antibodies against $\alpha 2$ integrin receptor were prepared by papain cleavage of protein G–Sepharose-bound IgG (Coulter and

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		Harris, 1983).
20	Wong GK <i>et al.</i> (1996)	Protein concentrations were estimated by measuring OD <sub>280</sub> using an extinction coefficient of 1.44 (Mandy and Nisonoff, 1963; Coulter and Harris, 1983) for both whole antibody and Fab fragments.
21	Balthasar JP <i>et al.</i> (1996)	Anti-MTX Fab fragments were prepared and purified from anti-MTX IgG following the procedure of Coulter and Harris, with slight modification. <sup>21</sup>
22	Krop I <i>et al.</i> (1996)	Fab rabbit anti-mouse <i>p</i> was prepared from purified IgC (Jackson ImmunoResearch Laboratories) using immobilized papain (Pierce, Rockford, IL) (44), and biotinylated with NHS (N-hydroxysuccinimide)-biotin (Zymed, San Francisco, CA). LFA-1 Ab I21/7 (rat IgG2a) was obtained from Life Technologies.
23	Kolodiej SJ <i>et al.</i> (1996)	Fab fragments were prepared by digestion with papain essentially as described (Coulter and Harris, 1983) using a 100:1 (w:w) ratio of 6E8 to papain.
24	D'Cruz OJ <i>et al.</i> (1995)	The protein concentrations of dialyzed IgG and <i>Fab</i> fractions were determined spectrophotometrically with values of E <sup>1%</sup> = 14.0 and 14.8 respectively (Coulter and Harris, 1983)
25	Agner AE <i>et al.</i> (1995)	Papain fragmentation of IgG produces an amino end Fab fragment that retains its antigen-binding capabilities, has low nonspecific binding, which does not crosslink to antigens and will not precipitate or clump antigen-antibody complexes (Coulter and Harris, 1983).
26	Porta C <i>et al.</i> (1994)	A method employing papain attached to beaded agarose (Sigma Chemical Co.) and adapted from the described by Coulter and Harris (1983) was used.
27	Kamihira M <i>et al.</i> (1994)	The Fab and Fc fragments were prepared according to the conventional method (10) using solid-phase papain and protein A column chromatography.
28	Otteson EW <i>et al.</i> (1994)	Fab fragments of mAb 439 were prepared by digestion with papain (24). The Fab and Fc fragments were separated on an immobilized protein A column (Pierce Chemical Co.) (25).
29	Zhang HF <i>et al.</i> (1993)	The protein concentrations of dialyzed IgG and <i>Fab</i> fractions were determined spectrophotometrically with values of E <sup>1%</sup> = 14.0 and 14.8 respectively (Coulter and Harris, 1983)
30	Gilbert MS <i>et al.</i> (1992)	Total IgG was first purified using a protein A column (Pierce, Rockford, IL), and the purified IgG was cleaved with papain (Coulter and Harris, 1983) to obtain Fab fragments.
31	Sutor GC <i>et al.</i> (1992)	Papain cleavage of rabbit anti-Id was performed according to Coulter and Harris (23). with some modifications.
32	Jons T <i>et al.</i> (1992)	F(ab) fragments were prepared by papain cleavage (Coulter and Harris, 1983).
33	Mahanthappa K <i>et al.</i> (1992)	Fab fragments were generated by incubation of the purified antibodies with immobilized papain (Pierce Chemical) by the method of Coulter and Harris (1983) and again purified by protein A-Sepharose 4B chromatography.
34	Ruf W <i>et al.</i> (1991)	Fab fragments of the Mabs....were produced by cleavage of the purified IgG with immobilized papain (29).
35	Ruf W <i>et al.</i> (1991)	Fab fragments of mAbs were produced by cleavage of purified IgG with immobilized papain (16)....
36	Pikuleva IA <i>et al.</i> (1991)	The degree of modification was determined spectrophotometrically, using the molar extinction coefficient....for IgG [19].
37	Taylor FB <i>et al.</i> (1991)	Fab fragments of TF9-5B7 were prepared by Coulter <i>et al.</i> [19].
38	Rock P <i>et al.</i> (1991)	Fab fragments were prepared from rabbit polyclonal anti-Forssman and anti-asialo-GM <sub>1</sub> IgG by the method of Coulter and Harris (1983) and dialyzed against 10 mM phosphate/ 10 mM NaCl buffer, pH 7.4.
39	Rock P <i>et al.</i> (1990)	Fab fragments of IgG fractions were prepared by using established procedures (Coulter & Harris, 1983).

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40	Bessen D <i>et al.</i> (1990)	The eluant was dialyzed, concentrated, and digested with immobilized papain overnight at 37°C with rotation (24) (Pierce).
41	Pikuleva IA <i>et al.</i> (1989)	<b>(Translated from Russian)</b> IgG from normal rabbit plasma was obtained by fractioning with ammonium sulphate, with subsequent cellulose chromatography DE-32 [6]. A mixture of unpurified activated derivatives of hemin in methanol was added to a 0.3 ml (0.55 mg/ml) solution of IgG in 20mM phosphate buffer, pH 7.2, titrated until a required level with pH imidazol. The final concentration of methanol did not exceed 3%. Upon the completion of the reaction, which was conducted in the dark, IgG was precipitated with acidic acetone (0.1ml HCl per 50 ml of acetone); the precipitate was then washed twice with acidic acetone, then with H <sub>2</sub> O. After that the precipitate was diluted in 1.0 ml of 0.1M phosphate buffer containing 6 M chlorhydrate guanide, pH 7.2, after with the absorption spectra were recorded. For calculations of the degree of modifications, the following values of molar absorption coefficients were used: for hemin $\epsilon_{390}$ 93 000 M <sup>-1</sup> . cm <sup>-1</sup> , $\epsilon_{280}$ 31,000 M <sup>-1</sup> . cm <sup>-1</sup> [2], for IgG $E_{280}^{1\%}$ 14 [7].
42	Dromer F <i>et al.</i> (1989)	Fab and Fc fragments were prepared according to Coulter & Harris (1983) using immobilized papain (Pierce Chemical Co., Rockford, IL).
43	Turco J <i>et al.</i> (1989)	IgG concentration was determined using the value $E_{1\%279nm} = 14.0$ . <sup>21</sup>
44	Hursting MJ <i>et al.</i> (1989)	Fab were prepared from this IgG fraction by papain digestion at 37°C using 2% papain (Type IV, Sigma Chemical Co., St. Louis, MO), 4 mmol/L EDTA, and 20 mmol/L cysteine with gentle agitation (7).
45	Das A <i>et al.</i> (1989)	Univalent antibody (Fab) was prepared from purified IgG according to the method of Coulter and Harris. <sup>18</sup>
46	Guzov VM <i>et al.</i> (1989)	The IgG concentration was determined spectrophotometrically, using the coefficient of extinction $A_{279\ 1\%} = 14.0$ [10].
47	Ketsary A <i>et al.</i> (1989)	The concentrations of IgG and F(ab') <sub>2</sub> fractions were determined by their A <sub>279</sub> (2).
48	Sakaguchi DS <i>et al.</i> (1989)	Monovalent fragments were generated using procedures described by Coulter and Harris (1983).
49	Savenkova MI <i>et al.</i> (1989)	<b>(Translated from Russian)</b> Optimization of usage of immunosorbents in immuno-ferment analysis (IFA) of strophanthin K Solid phase immunosorbents have been used in heterogenous IFA for detection of free and bound antigens. For synthesis of the immunosorbents, activated sepharose is often used: BrCN-sepharose for immobilizing Ig [10], CH-sepharose for immobilizing Fab-fragment antibodies [8], and thiol-sepharose for immobilizing second antibodies [11].
50	Wessels MR <i>et al.</i> (1989)	Fab fragments were prepared from protein A-purified IgG by papain cleavage using papain linked to agarose beads (Sigma Chemical Co.), as described (13).
51	Uggla CK <i>et al.</i> (1989)	The 3G8 F(ab') <sub>2</sub> fragment was obtained after papain cleavage and verified by sodium dodecyl sulphate (SDS) gel analysis [8].
52	Liu <i>et al.</i> (1989)	The Fab concentration was determined by absorbance to be 1 mg/ml using a value of $\epsilon = 14.8$ (21).
53	Schefner M <i>et al.</i> (1989)	For the preparation of fab fragments....(23,24)
54	Shima <i>et al.</i> (1988)	The Fab fraction of affinity-purified rabbit IgG was prepared using immobilized papain (Pierce Chemical Co.) followed by protein A-Sepharose chromatography essentially according to the method of Coulter and Harris (17).
55	Bizzini <i>et al.</i> (1988)	From immune sera, the IgG fraction was precipitated by ammonium sulfate at 50% saturation and was digested by papain (Coulter and Harris, 1983).
56	Newkirk MM <i>et al.</i> ( )	Fab fragments from human, rat, rabbit and mouse IgG have been made routinely by digesting the immunoglobulins with papain (1-5)
57	Sada E <i>et al.</i> (1989)	NO REFERENCE TO COULTER
58	Russell DG <i>et al.</i> (1986)	Anti-gp63 IgG was purified by protein A Sepharose chromatography and was

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		digested with immobilized papain (21)
59	Gawade S <i>et al.</i> (1985)	These fragments were prepared by papain digestion of IgG using immobilized Papain (8).
60	Mohanty JG <i>et al.</i> (1985)	According to the literature, polypeptide g corresponds to Fab fragments (7).
61	Fedinec AA <i>et al.</i> (1985)	Antibody F(ab) fragments: these fragments were prepared by papain digestion of IgG using immobilized papain (6).
62	Tseng J. <i>et al.</i> (1988)	Fab fragments of mAbs (IgG isotypes) and heterologous IgG were prepared by immobilized papain digestion (Coulter and Harris, 1983).